

REMARKS

Applicants submit this Amendment in response to the Office Action dated August 19, 2008. Reconsideration of the subject application as amended herein is respectfully requested.

Claim 1 has been amended so as to replace the phrase "relatively hydrophilic organic solvent having no negative effect on the reactivity of the lipase" with the word "tert-butanol"; claim 2 has been cancelled, without prejudice; and in claim 12, the phrase "said organic solvent added" has been amended to read "tert-butanol added as the organic medium"; thus, the scope of claims 1 and 12 has been narrowed.

In the Office Action, the Examiner rejected claims 1-14 under 35 U.S.C. §103(a) as unpatentable over National Research Inst of Brewing (JP2002-233393, hereinafter referred to as "Brewing"), in view of Siegfried et al. (WO 03004591) and Soumanou et al. (Enzyme and Microbial Technology, July 2003), and further in view of Wu et al (US6398707). Applicants respectfully contend, however, that amended claim 1 and its dependent claims are not obvious over the cited documents.

In any process for producing biodiesel from a short chain alcohol (such as methanol) and an oil, using immobilized enzyme as a catalyst, there is usually an

excess of the short chain alcohol in the reaction system, due to the poor miscibility of the short chain alcohol with the oil; this excess tends to cause the enzyme to become deactivated. In order to alleviate this "poisoning" effect of the short chain alcohol the short chain alcohol has to be added batchwise in a conventional solvent-free process, which renders the process unduly complicated.

Some researchers have used organic solvents, such as 1,2-dioxane and hexane, to alleviate the poisoning effect of the short chain alcohol on the enzyme. However, these organic solvents are highly hydrophobic. Although these hydrophobic organic solvents can alleviate the negative effect of the short chain alcohol on the lipase to some extent, essentially by diluting the oil, the activity of the lipase still decreases significantly during the recirculation process of the lipase. The main reason for the decrease in the activity of the lipase is that, during the reaction process, glycerin is formed as a by-product and it has negative effect on the lipase. Glycerin is strongly hydrophilic and it tends to absorb on the surface of the molecules of the lipase during the reaction process, and thus affect the activity of the lipase. When using a hydrophobic organic solvent (such as 1,4-dioxane or hexane) as the reaction medium, since glycerin has such poor miscibility with these solvents, almost all the glycerin produced in the reaction process ends up becoming attached to the surface of the molecules of the lipase, and thus greatly reduces the activity of the lipase.

In claim 1 of the present application, as amended, tert-butanol is used as the organic solvent of the transesterification reaction. Since both the short chain alcohol (such as methanol) and the by-product glycerin can be dissolved in tert-butanol very well, the negative effects of both the short chain alcohol and glycerin on the lipase can be prevented, and the lifetime of the lipase can be prolonged. The use of tert-butanol as the organic solvent in this reaction system is novel, and is neither taught nor suggested by the prior art.

Brewing describes a method for producing biodiesel from fats and oils using lipase through a transesterification reaction, and also mentions that such a reaction is possible in the presence of an organic solvent, such as DMSO, hexane, chloroform, etc. However, **Brewing does not disclose that tert-butanol is used as the solvent of the transesterification reaction.** Although Brewing mentions tert-butanol in paragraph [0042], it is mentioned there as one of the *starting materials* (reactants) of the transesterification reaction, *not* as a solvent; this is evident from the amount of the tert-butanol used, which is in a ratio of between 1:1 and 1:4 (by mole) to the fats and oils (see paragraph [0044] of Brewing), which is too low to act as a solvent.

Moreover, applicants would like to point out that according to Brewing, those solvents involved in the reaction system, such as DMSO, hexane and chloroform, are *not* purposely added as a reaction medium for producing biodiesel; instead, these

solvents are involved in the reaction system inadvertently, as impurities. It is well known in the art that the above-mentioned solvents are usually used industrially to extract oil raw materials. Due to different refining extents of the oil raw materials, the solvents for the extraction may not be removed completely. So, when using different oil raw materials for the production of biodiesel, there might be a little (5-15% as disclosed in Brewing) of above-mentioned organic solvents as impurities in the oil raw materials (see paragraphs [0043], [0046] and Table 3 of Brewing).

In comparison, the organic solvent tert-butanol used in the present invention is added purposely to act as a reaction medium, in order to dissolve the short chain alcohol starting material and the glycerin by-product so as to eliminate their negative effects on the lipase, thereby significantly increasing the stability of the lipase and prolonging the lifetime of the lipase by dozens of times. The solvents mentioned in Brewing are organic impurities caused by incomplete refining of the oil raw materials. Brewing does not teach or suggest the advantageous use of tert-butanol as a reaction medium.

Siegfried et al. discloses a method of the transesterification of fats and/or oils, in which at least one alkyl fatty acid ester is added in such an amount during the initial phase that the resulting reaction mixture is a single phase. However, Siegfried et al. does not teach or suggest the use of tert-butanol as the reaction medium of the

Wu et al. disclose a method of preparing biodiesel by transesterification of triglycerides and a short chain alcohol, wherein the immobilized lipase catalyst is swelled and/or cleaned by an alcohol with a carbon atom number not less than three, such as iso-propanol, 2-butanol and tert-butanol. However, applicants respectfully point out that the tert-butanol mentioned by Wu et al. **is not used as a reaction medium**. As mentioned in column 2 of Wu et al., a solvent, such as tert-butanol, is used to perform **an immersion pretreatment** on the immobilized lipase, or to wash the immobilized lipase so as to increase the activity of a commercially available immobilized lipase or to

regenerate a deactivated immobilized lipase.

After the immersion pretreatment, the lipase is used as a catalyst to produce biodiesel from an oil and a C_1 - C_3 alcohol in a solvent-free system (see column 2 line 66 to column 3 line 44 of Wu et al.). Since the production of the biodiesel is still carried out in a solvent-free system, the addition of the C_1 - C_3 alcohol has to be strongly controlled (*i.e.*, not more than 3 moles should be used; see column 4 lines 47-49 of Wu et al.), and the C_1 - C_3 alcohol has to be added batchwise.

The present invention is distinguished from Wu et al. in that, in present invention, tert-butanol is used as reaction medium and the negative effects of both the short chain alcohol starting material and the by-product glycerin on the lipase can be prevented. The molar ratio of the short chain alcohol to the oil in the reaction system is up to 6, and all of the short chain alcohol can be added into the reaction system at once.

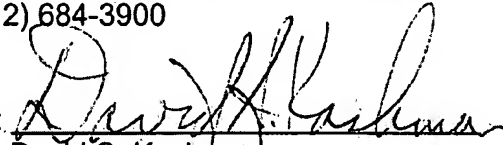
In summary, none of the cited documents teach or suggest the subject matter of amended claim 1. With regard to the use of the hydrophilic and polar organic solvent tert-butanol as the reaction medium, these cited documents even teach away. Therefore, the amended claim 1 and its dependent claims of the present invention are not obvious over all of the cited documents.

Applicants have responded herein to the points raised by the Examiner in the Office Action, and applicants have amended the claims in an earnest effort to place this application in condition for allowance. Accordingly, further favorable action in connection with this patent application is earnestly solicited. The Examiner is invited to contact the undersigned attorney by telephone if it will advance the prosecution of this case.

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